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invited review

Cardiovascular and renal control in NOS-deficient mouse models

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Ortiz, Pablo A., and Jeffrey L. Garvin. Cardiovascular and renal control in NOS-deficient mouse models. *Am J Physiol Regul Integr Comp Physiol* 284: R628–R638, 2003; 10.1152/ajpregu.00401.2002.—Nitric oxide (NO) plays an essential role in the maintenance of cardiovascular and renal homeostasis. Endogenous NO is produced by three different NO synthase (NOS) isoforms: endothelial NOS (eNOS), inducible NOS (iNOS), and neuronal NOS (nNOS). To investigate which NOS is responsible for NO production in different tissues, NOS knockout (−/−) mice have been generated for the three isoforms. This review focuses on the regulation of cardiovascular and renal function in relation to blood pressure homeostasis in the different NOS−/− mice. Although regulation of vascular tone and cardiac function in eNOS−/− has been extensively studied, far less is known about renal function in these mice. eNOS−/− mice are hypertensive, but the mechanism responsible for their high blood pressure is still not clear. Less is known about cardiovascular and renal control in nNOS−/− mice, probably because their blood pressure is normal. Recent data suggest that nNOS plays important roles in cardiac function, renal homeostasis, and regulation of vascular tone under certain conditions, but these are only now beginning to be studied. Inasmuch as iNOS is absent from the cardiovascular system under physiological conditions, it may become important to blood pressure regulation only during pathological conditions related to inflammatory processes. However, iNOS is constitutively expressed in the kidney, where its function is largely unknown. Overall, the study of NOS knockout mice has been very useful and produced many answers, but it has also raised new questions. The appearance of compensatory mechanisms suggests the importance of the different isoforms to specific processes, but it also complicates interpretation of the data. In addition, deletion of a single gene may have physiologically significant effects in addition to those being studied. Thus the presence or absence of a specific phenotype may not reflect the most important physiological function of the absent gene.

endothelial nitric oxide synthase; neuronal nitric oxide synthase; inducible nitric oxide synthase; knockout mice; blood pressure

NITRIC OXIDE (NO) plays an important role in the maintenance of cardiovascular and renal homeostasis. In the cardiovascular system, it is involved in regulation of vascular tone (22), cardiac contractility (67), cell growth (76), vascular remodeling (49), and baroreflex function (7, 48, 111). In the kidney, NO regulates salt and fluid reabsorption (63, 89), hemodynamics (44), renin secretion (46), and tubuloglomerular feedback (TGF; 73, 106, 107). Most of these processes are important in both short- and long-term regulation of arterial blood pressure and have been extensively studied in the last 10 years.

Endogenous NO is enzymatically produced from conversion of the amino acid L-arginine to L-citrulline, a reaction catalyzed by the enzyme NO synthase (NOS). Three different NOS isoforms have been cloned and characterized: neuronal NOS (nNOS), endothelial NOS

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(eNOS), and inducible NOS (iNOS). The three isoforms are differentially expressed throughout the cardiovascular system and the kidney, and one or more isoforms can be expressed in the same cell type. To answer important questions regarding which isoforms produce the NO that regulates physiological processes, mice with disruption of each of the NOS genes have been generated. Studies of the different NOS knockout (−/−) mice have provided many answers but have also raised new questions regarding the role of the various NOS isoforms. Many reviews have concentrated on different pathophysiological aspects studied in NOS−/− mice (12, 25, 34, 35, 50, 78, 93). This review will focus on recent data concerning the regulation of cardiovascular and renal function in relation to blood pressure homeostasis in the different NOS−/− mice.

eNOS−/−

Blood pressure. Given the location of e, n, and iNOS expression in the cardiovascular and renal systems, NO produced by each isoform has the potential to alter blood pressure. Most studies have shown that eNOS−/− mice have higher blood pressure than wild-type mice, although the magnitude of hypertension reported by different laboratories varies. Differences in systolic arterial pressure in conscious eNOS−/− compared with wild-type mice range from 20 (45) to 50 mmHg (85, 86), while differences in mean blood pressure in anesthetized mice range from 14 (5) to 37 mmHg (101). The varying magnitude of the hypertension observed in eNOS−/− may be due to the use of different methods for measuring blood pressure or the genetic backgrounds of the strains used. However, even with these differences, eNOS−/− mice were found to be hypertensive in all cases.

Arterial blood pressure is the product of cardiac output and total peripheral resistance. In turn, cardiac output depends on heart rate, cardiac contractility, and total extracellular fluid volume, which is maintained by the kidney; peripheral resistance is controlled by the tone of resistance vessels. It is frequently assumed that all hypertension in eNOS−/− is caused by the lack of endothelium-derived NO and the resulting increase in arterial tone and peripheral resistance. However, this simple explanation is not fully supported by the data, as discussed below.

Vascular function. The amount of eNOS expressed in the vascular endothelium, together with the data showing that NO is an important endothelium-derived relaxing factor, indicates that eNOS-derived NO is essential to regulation of vascular tone (22). Thus the hypertension observed in eNOS−/− can be partly attributed to increased vascular resistance caused by the lack of endothelial NO. Several investigators have studied the response of eNOS−/− arteries to vasodilator stimuli. It was first reported that aortic rings isolated from eNOS−/− do not relax in response to ACh (36). Lamping and Faraci (47) observed a complete lack of ACh-induced relaxation in carotid artery rings, with no difference between male and female mice. To verify that eNOS mediates ACh-induced relaxation in carotid arteries, Scotland et al. (80) transfected the endothelium of eNOS−/− mouse carotid arteries with an adenoviral vector carrying the gene for eNOS and in this way restored ACh-induced relaxation. These data indicate that *1*) in large vessels, such as the aorta or carotid artery, eNOS mediates ACh-induced vasodilatation and *2*) there is no compensatory vasodilator mechanism for ACh.

Despite the role of eNOS in major arteries, vascular peripheral resistance is primarily controlled by small resistance arterioles. In contrast to large arteries, other endothelium-derived vasodilators have been shown to compensate for the lack of eNOS in resistance arteries. Myogenic responses and ACh-induced relaxation are preserved in mesenteric arteries of eNOS−/−. These responses could be prevented by K^+ channel blockers in eNOS−/− but not in wild-type mice, suggesting that they are mediated by an endothelium-derived hyperpolarizing factor (EDHF) (18, 79). Sun et al. (91) reported that in gracilis muscle arterioles of male eNOS−/−, flow-induced dilatation was completely blocked by indomethacin, whereas this drug only inhibited 50% of the response in wild-type mice. Interestingly, the same group reported that in female eNOS−/−, a different vasodilator mechanism compensates for the lack of eNOS. In females, flow-induced vasodilatation was not prevented by indomethacin but was completely blocked by a Ca^{2+}-activated K^+ channel blocker or a cytochrome P450 inhibitor (31). A gender difference in the relative contribution of NO to endothelium-dependent vasodilatation has been reported in wild-type mice and in rats (31, 32, 55, 91). Thus a different compensatory mechanism could be activated in the absence of eNOS in male or female mice. However, the precise explanation for these differences is still not known.

On the basis of these data, it is possible to conclude that eNOS-derived NO is an important mediator of vasodilator stimuli that affect vascular tone in most arteries studied. In large arteries the lack of endothelial NO in eNOS−/− impairs the relaxant effect of vasodilators, and this is apparently not compensated for by other endothelial vasodilators. In resistance arteries, the lack of eNOS is sometimes compensated for by EDHF, a cyclooxygenase product, or by nNOS-derived NO, as in the brain pial arterioles and the coronary microcirculation (33, 56) (Fig. 1). It is important to note that in some cases such as the pulmonary circulation, the lack of eNOS is not compensated for at all (20). Thus most data indicate that the mechanisms that compensate for the lack of eNOS-derived NO are specific to different vascular beds, and one or more mechanisms may be activated to regulate arterial tone in the absence of eNOS. Although the absence of eNOS appears to be compensated for in most resistance arteries, eNOS−/− mice exhibit higher blood pressure and it is still not known whether they have increased total peripheral resistance. To us, this raises the questions of how much of the hypertension observed in eNOS−/− is caused by an increase in vascular tone

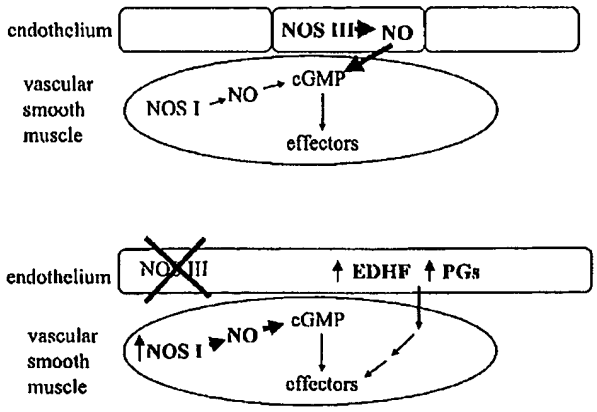

Fig. 1. Vasodilator mechanisms that compensate for the lack of endothelium-derived nitric oxide (NO) in resistance arterioles of endothelial NO synthase knockout (eNOS−/−) mice. EDHF, endothelium-derived hyperpolarizing factor.

and whether other mechanisms such as increased cardiac output and increased salt and fluid absorption by the kidney may be involved in the hypertension exhibited by these mice.

Renal function. Extracellular fluid volume is regulated by the kidney, where eNOS is expressed in the endothelium of the renal vasculature, including the afferent and efferent arterioles and vasa recta. eNOS is also present in proximal tubules, thick ascending limbs, and collecting ducts (44, 96). However, despite the important role of NO in the regulation of various physiological processes in the kidney, little is known about the contribution of the three NOS isoforms to these mechanisms.

NO is known to be involved in the regulation of renin secretion. However, the data are still contradictory, with some reports concluding that endothelial NO stimulates renin secretion, whereas others found the opposite (46). In eNOS−/−, a decrease in total kidney renin mRNA was observed, whereas plasma renin levels were elevated by 50% compared with wild-type mice despite the high blood pressure (81). These data suggest that eNOS-derived NO tonically inhibits renin release and that regulation of renin release by renal perfusion pressure is impaired because renin levels would be low due to the high blood pressure. Wagner et al. (101) found a decrease in renin mRNA levels in total kidney homogenates but also reported lower levels of renin activity in the eNOS−/− kidney. Although this latter result contradicts the increase in plasma renin observed by others, total renin activity does not necessarily reflect plasma levels or rate of renin secretion. In a recent study, Beierwaltes et al. (5) found no difference in plasma renin content between eNOS−/− and wild-type mice. They also found that in anesthetized mice, renin secretion in response to reduced perfusion pressure was normal in eNOS−/−, whereas acute inhibition of NOS completely prevented pressure-dependent renin release in wild-type mice. The authors concluded that pressure-dependent renin release is completely compensated for in eNOS−/−. Inasmuch as NO derived from macula densa nNOS regulates renin secretion, it is possible that this pathway is upregulated in eNOS−/−. It is important to note that plasma renin levels have been found to be either equal or increased in eNOS−/− in the context of increased blood pressure (5, 81), suggesting that this mechanism may contribute to hypertension. The precise role of NO in the regulation of renin secretion is still unknown, as are the molecular mechanisms by which NO acts in renin-secreting cells.

Previous in vivo and in vitro data support a role for endothelium-derived NO in the regulation of renal vascular tone (44). In eNOS−/−, basal renal perfusion pressure was increased, but renal blood flow was similar to wild-type mice (5). Acute infusion of the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) decreased renal blood flow in wild-type mice but had no effect in eNOS−/−. These data suggest that in the renal vasculature compensatory mechanisms are activated in the absence of eNOS to maintain normal renal blood flow. However, the mechanism by which renal blood flow is maintained is not known. In rabbit afferent arterioles, NOS inhibition reduced basal arteriolar diameter (38, 43). In contrast, Patzak et al. (66) reported that in isolated perfused afferent arterioles from wild-type mice, neither L-arginine nor L-NAME changed basal arteriole diameter, suggesting that basal NO activity is negligible in this preparation. However, angiotensin II-induced contractions were greatly enhanced in afferent arterioles of eNOS−/−, suggesting that eNOS-derived NO decreases the constrictor response to angiotensin II. Although NO has been shown to modulate the vasoconstrictor response to other hormones, the role of eNOS-derived NO in these responses has not been studied to our knowledge.

NO is an important regulator of the renal medullary microcirculation (53, 65). Medullary infusion of the nonspecific NOS inhibitor L-NAME decreases medullary blood flow and increases sodium retention and blood pressure (54). Conflicting results have been published regarding a role for nNOS in this process. It was first shown that infusion of anti-sense oligonucleotides for nNOS into the medullary interstitium caused salt-sensitive hypertension in rats (51, 52), suggesting a role for nNOS in blood flow regulation. However, infusion of selective nNOS inhibitors into the renal medulla did not affect medullary blood flow, although it decreased NO levels (41), suggesting that nNOS does not play a role in medullary blood flow regulation. Thus the particular NOS isoform responsible for regulation of medullary blood flow or the cell type where the NO that affects blood flow is produced is unknown. In the renal medulla, NO could be produced by the thick ascending limb (62), descending vasa recta (75), or medullary collecting duct (11, 57). Despite the important role of NO in the medullary microcirculation, NOS knockout mice have not yet been used to study this physiological process.

NO has been shown to inhibit NaCl and fluid reabsorption along the nephron and promote renal sodium

and water excretion; however, very little is known about the role of endogenous NO and the contribution of the different NOS isoforms to this process (63). We reported that in the rat thick ascending limb, L-arginine stimulates endogenous NO production and decreases NaCl and $NaHCO_3$ reabsorption (60–62, 70). To investigate which NOS isoform mediates this response, we studied the effect of L-arginine on thick ascending limbs from eNOS−/−, iNOS−/−, and nNOS−/− mice. We found that in thick limbs from wild-type, nNOS−/−, and iNOS−/− mice, L-arginine inhibited NaCl absorption, whereas it had no effect in eNOS−/−. A NO donor was able to inhibit NaCl transport in eNOS−/−, indicating that the second messenger cascade for NO was intact (69). These data suggest that eNOS-derived NO inhibits NaCl absorption in this nephron segment and that the lack of eNOS is not compensated for by other NOS isoforms.

Although the proximal tubule and collecting duct also express eNOS, little is known about basal or stimulated reabsorption rates in these tubular segments from eNOS−/−. In proximal tubules microperfused in vivo, Wang (103) reported no differences in basal fluid and bicarbonate reabsorption rates in eNOS−/− compared with wild-type mice. In contrast, Adler et al. (1) reported that basal oxygen consumption in cortical renal slices was higher in eNOS−/− than in wild-type mice. The renal cortex is composed mostly of proximal tubules and a minor fraction of vascular cells, distal tubules, and cortical thick ascending limbs. Because the basal metabolic rate of epithelial cells is much higher than that of vascular cells, and because NO has been shown to inhibit proximal tubule sodium reabsorption (63), the higher rate of oxygen consumption found in eNOS−/− is likely due to increased basal rates of sodium reabsorption by the proximal tubule, caused by the lack of eNOS-derived NO. However, the precise role of eNOS in the proximal tubule is currently unknown. Thus it is possible that a lack of eNOS-derived NO may chronically increase reabsorption of NaCl by the nephron and increase TGF responses, contributing to the hypertension observed in eNOS−/− (Fig. 2).

Cardiac function. Elimination of NO produced by eNOS in the heart may also enhance cardiac output and contractility. Recently, a large number of publications have centered on the role of NO in regulation of cardiac function. In the heart, eNOS is expressed not only in the endothelium of the coronary vessels but also in cardiac myocytes. In addition to eNOS, cardiac myocytes have also been shown to express nNOS in the mitochondria and sarcoplasmic reticulum (42, 110), suggesting that NO plays an important role in myocyte physiology.

Most investigators have reported that the basal heart rate in conscious eNOS−/− mice is significantly lower than in wild-type mice (23, 45, 81, 85, 110). However, others have reported that heart rates measured in anesthetized animals were no different between eNOS−/− and wild-type mice (27, 36). An acute increase in blood pressure decreases the heart rate via a baroreflex mechanism. However, during long-term increases in blood pressure, the baroreceptor resets to the new pressure and the heart rate returns to baseline. In eNOS−/−, the increase in blood pressure is chronic and thus the heart rate would be expected to be normal. Although there is still no explanation for the decreased heart rate observed in conscious eNOS−/−, it is possible that eNOS-derived NO can affect baroreflex resetting or be involved in establishing the baroreceptor setpoint (30, 48, 84). In addition, the fact that the heart rate is not different in anesthetized mice may reflect the loss of baroreflex influence caused by the

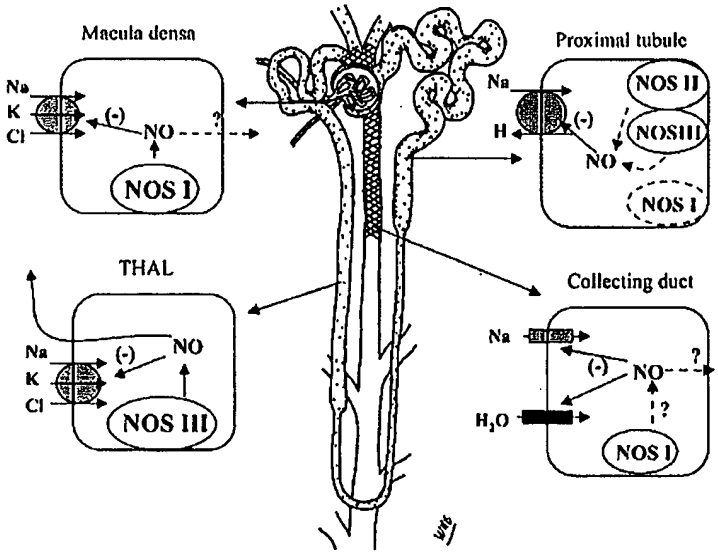

Fig. 2. Role of the different NOS isoforms along the nephron. Dashed line indicates no direct experimental evidence.

anesthesia. Overall, it is still not clear why eNOS−/− have a lower heart rate than wild-type mice.

In eNOS−/− mice, basal parameters of cardiac function in vivo appear to be normal. Assessment of cardiac function by echocardiography showed no significant differences in left ventricular shortening fraction, ejection fraction, and cardiac output between eNOS−/− and wild-type mice (110). Basal systolic contractility, reflected by the maximum rate of pressure development (dP/dt_{max}), was no different from wild-type mice as measured with an intraventricular pressure catheter (27). An increase in left ventricular mass and posterior wall thickness together with an increase in myocyte size indicative of cardiac hypertrophy have been observed in eNOS−/− (110). Although these changes are most likely due to hypertension, a role for NO in cardiac myocyte growth cannot be ruled out.

Basal cardiac contractility was found to be similar between eNOS−/− and wild-type mice. However, data obtained from perfused whole heart (Langendorff) preparations or in vivo suggest that during β-adrenergic stimulation cardiac contractility is influenced by eNOS-derived NO. Gyurko et al. (27) found that in an isolated perfused whole heart preparation, isoproterenol-stimulated cardiac contractility was enhanced in hearts from eNOS−/−, whereas β-adrenergic receptor density was no different between eNOS−/− and wild-type mice. In vivo assessment of cardiac function showed that isoproterenol-stimulated cardiac contractility was enhanced in eNOS−/− compared with wild-type mice. The effect of isoproterenol in eNOS−/− was similar to the response observed in wild-type mice treated with the NOS inhibitor L-NNA, supporting a role for eNOS in mediating this effect (27). Another group of investigators also reported that isoproterenol-stimulated cardiac contractility was enhanced in hearts from eNOS−/− mice (100). These data indicate that in the intact animal, eNOS-derived NO modulates the systolic response to β-adrenergic stimulation.

The mechanism by which eNOS modulates β-adrenergic-stimulated cardiac contractility appears to involve activation of β_3-adrenergic receptors. It has been shown that in β_3-adrenergic receptor knockout mice (β_3−/−), the contractile response to isoproterenol is enhanced to the same extent as in eNOS−/− (100). In addition, NOS inhibitors enhance the β-adrenergic inotropic response in wild-type mice but not in β_3−/−. These data suggest that binding of isoproterenol to the β_1- and β_2-adrenergic receptors stimulates cardiac contractility, whereas simultaneous binding to the β_3 receptor modulates the positive inotropic effect by activating eNOS. A recent report confirms the latter hypothesis by showing that a selective β_3-receptor agonist decreased intracellular calcium and sarcomere length in wild-type cardiac myocytes but failed to produce this effect in myocytes from eNOS−/− (4). However, the mechanism for the increase in intracellular calcium observed in eNOS−/− mice remains unclear.

Whereas most data support a role for eNOS in decreasing β-adrenergic effects on contractility, in vitro studies using isolated myocytes are more controversial. Han et al. (28) first reported that in isolated cardiac myocytes from eNOS−/−, the muscarinic agonist carbachol failed to reverse isoproterenol-stimulated contractions. These authors showed that carbachol failed to decrease isoproterenol-stimulated L-type Ca^{2+} channel activation in eNOS−/− mice, whereas it completely abolished the effect of isoproterenol in myocytes from wild-type mice. Although these data are consistent with eNOS-derived NO blunting β-adrenergic stimulation of contractility, others have failed to reproduce these results. Vandecasteele et al. (99) found no difference in β-adrenergic-induced contractility of isolated papillary muscles between eNOS−/− and wild-type mice. They also reported that carbachol blocks isoproterenol-stimulated L-type Ca^{2+} channel currents to the same extent in isolated eNOS−/− and wild-type myocytes. In agreement with the last report, two other groups of investigators found no differences in isoproterenol-stimulated L-type Ca^{2+} channel currents or the inhibitory effect of muscarinic receptor agonists between isolated eNOS−/− and wild-type myocytes (6, 24). Consistent with a role for eNOS in decreasing the β-adrenergic increase in contractility, Barouch et al. (4) found that the isoproterenol-stimulated increase in intracellular calcium was enhanced in myocytes from eNOS−/−. Contractility data obtained from isolated myocytes are still unresolved. The different results observed in isolated cell preparations may be attributable to blockade or activation of second messenger cascades caused by physical disruption of the tissue or the different protocols used to obtain it. Although the data suggest that eNOS-derived NO affects intracellular calcium balance, the mechanism involved is still unclear.

In summary, in vivo and in vitro data indicate that, under basal conditions, cardiac function is normal in eNOS−/−, suggesting that the lack of eNOS is compensated for by other mechanisms or that eNOS only plays a minor role in basal cardiac function. However, in vivo data indicate that when sympathetic output and adrenergic discharge are increased, eNOS mediates the negative inotropic effect caused by stimulation of the β_3-adrenergic receptor. Thus eNOS-derived NO appears to be an important physiological modulator of cardiac contractility.

Finally, genetic deletion of eNOS may disrupt the function of other important regulators of blood pressure by affecting central nervous system activity. For example, Stauss et al. (85, 87) found that blood pressure is more variable in eNOS−/− compared with wild-type mice, suggesting that baroreflex responses are blunted in the former. Still, little is known about other central nervous system effects of deleting eNOS, in particular regarding blood pressure control.

Studies in eNOS−/− mice have clearly demonstrated that NO produced by eNOS plays an important role in the regulation of blood pressure. Although one might expect that eliminating eNOS from the vasculature would play a predominant role in the hypertension seen in eNOS−/− mice, other vasodilators partially compensate for the loss of eNOS in resistance

vessels. Thus the hypertension observed in these mice can also be attributed to the lack of eNOS-derived NO in the kidney and heart.

nNOS−/−

Blood pressure. In contrast to the findings observed in eNOS−/−, blood pressure in nNOS−/− mice has generally been shown to be similar to that of wild-type controls (4, 40, 59, 98). These data suggest that genetic deletion of nNOS is compensated for, in terms of blood pressure regulation. Alternatively, the hypotensive actions of nNOS may be counterbalanced by its hypertensive effects. Acute administration of the nNOS inhibitor 7-nitroindazole (7-NI) to eNOS−/− mice significantly reduced blood pressure (45), suggesting that nNOS contributes to their hypertension. However, Barouch et al. (4) reported that systolic blood pressure in mice deficient in both eNOS and nNOS (e-nNOS−/−) was higher than in wild-type mice and similar to eNOS−/−. Although nNOS may be antihypertensive in some cases (74, 106), in others it appears to be prohypertensive (4, 45). Thus the role of nNOS in the global regulation of blood pressure is still not well defined, and more work is needed in this area.

Vascular function. The data showing that blood pressure in nNOS−/− is similar to that of wild-type mice suggest that nNOS does not play an important role in the regulation of basal vascular tone. However, it has been shown that nNOS is expressed in vascular smooth muscle cells (10, 33) and also in cardiac myocytes (110). Therefore, while vascular tone is regulated by eNOS under basal conditions, it is possible that when eNOS-dependent vasodilatation is impaired, nNOS-derived NO could modulate vascular tone. For example, in brain pial arterioles of eNOS−/−, ACh-induced dilatation was found to be reduced by only 25% compared with wild-type controls. In the presence of the nNOS inhibitor 7-NI, ACh-induced dilatation was reduced by 50% in eNOS−/− but normal in wild-type mice, suggesting that nNOS-derived NO compensates for the lack of eNOS (56). This is supported by Huang's study (33), showing that in isolated perfused coronary arterioles of eNOS−/−, which exhibited normal flow-induced dilatation, 7-NI blunted flow-induced dilatation by 40% but had no effect in wild-type arterioles. In addition, these authors found upregulation of nNOS expression in the endothelium and smooth muscle of coronary arteries. Brandes et al. (9) observed increased sensitivity of soluble guanylate cyclase in aortic rings of eNOS−/−. These data suggest that despite the small amount of nNOS in blood vessels, low levels of nNOS-derived NO could compensate for the lack of eNOS in these mice. To date, compensation by nNOS has only been evident in eNOS−/− arterioles, but it could also be important in other conditions where endothelium-dependent vasodilatation is impaired, such as hypercholesterolemia (8, 90) and diabetes (16).

Renal function. In the kidney, nNOS is expressed in macula densa cells, collecting ducts (77, 105, 107), and in thick ascending limbs (Garvin, unpublished observations). nNOS expression is significantly higher in the macula densa compared with other tubular cells, suggesting an important role for this isoform in modulating the function of the macula densa and juxtaglomerular apparatus (106). In fact, studies using pharmacological inhibitors of NOS have shown that nNOS-derived NO produced in the macula densa blunts TGF responses in rats, rabbits, and mice (74, 95, 98). In wild-type mice, inhibition of macula densa nNOS with 7-NI increases the magnitude of the TGF response, seen as greater constriction of the afferent arteriole (74). Vallon et al. (98) studied TGF responses in nNOS−/− in vivo by monitoring changes in proximal stop-flow pressure while increasing luminal perfusion rates to the distal nephron. They found no difference in TGF responses between nNOS−/− and wild-type mice; however, blocking NOS with L-NNA increased TGF in wild-type mice but had no effect in nNOS−/−. In agreement with this report, we found no difference in TGF responses between nNOS−/− and wild-type mice. However, 7-NI potentiated TGF responses in wild-type mice, whereas it did not affect TGF in nNOS−/− (74). Taken together, these data suggest that in wild-type mice, nNOS-derived NO produced in the macula densa blunts TGF. However, chronic deletion of nNOS is compensated for by some mechanism that helps maintain glomerular hemodynamics. Because we have shown that NO produced by the thick ascending limb (presumably by eNOS) can also blunt TGF responses (102), it could be that this mechanism is upregulated in nNOS−/− to maintain normal TGF.

The precise mechanism by which nNOS-derived NO attenuates the TGF response is currently unknown. The published data allow for speculation on some possible mechanisms that could mediate the effects of NO in TGF. Data from our laboratory have shown that inhibition of soluble guanylate cyclase or protein kinase G in the macula densa, but not in the afferent arteriole, blunts TGF similarly to inhibition of nNOS (73). We have also shown that in the thick ascending limb NO decreases the activity of the apical Na-K-2Cl cotransporter, which is also located in the macula densa and is known to initiate TGF (64). Thus one possible mechanism is that NO acts in an autocrine manner in the macula densa, blunting TGF by tonically inhibiting NaCl entry via the Na-K-2Cl cotransporter. Other mechanisms may involve the inhibition of 5′-ectonucleotidase by NO. Adenosine is a mediator of the TGF response (68, 71, 92, 94), and it could be released by the macula densa or produced in the interstitium by enzymatic degradation of ATP, ADP, and AMP by 5′-ectonucleotidase. Because NO has been shown to inhibit this enzyme (82), it is possible that NO blunts TGF by tonically inhibiting it and thus decreasing adenosine levels. Another possibility is that NO produced in the macula densa diffuses through the interstitial space and activates soluble guanylate cyclase in smooth muscle cells, increasing cGMP levels and dilating the afferent arteriole. Finally, new mechanisms could be hypothesized in view of recent data

showing that intact extraglomerular mesangial cells and their gap junctions are necessary for TGF (72).

nNOS has also been shown to be expressed in the collecting duct (77, 105), though at lower levels than in macula densa cells. Although NO has been found to inhibit sodium and fluid transport in this segment (63), to our knowledge there are no studies regarding the contribution of nNOS to this effect. There is still no evidence for functional expression of nNOS in the proximal tubule, and the effects of NO on proximal tubule transport remain controversial. In vivo data have shown that NO can inhibit and stimulate sodium and fluid reabsorption, while most in vitro data are consistent with NO inhibiting transport in this segment (63). In support of an inhibitory effect of endogenous NO on proximal tubule transport, Vallon et al. (98) observed that in vivo microperfused proximal tubules of nNOS−/− exhibited higher fluid and chloride absorption rates compared with proximal tubules of normal mice, suggesting that endogenously produced NO inhibits proximal tubule transport. In contrast to the study of Vallon et al. (98), Wang et al. (104) reported that nNOS knockout mice exhibited lower fluid and bicarbonate absorption rates than proximal tubules from wild-type mice, suggesting that NO produced by nNOS stimulates rather than inhibits transport in the proximal tubule.

The explanation for the disparate results regarding the role of nNOS-derived NO in proximal tubule transport is unclear. However, in nNOS−/− mice, nNOS is genetically deleted from all tissues, not just the proximal tubule. Thus the difference between wild-type and nNOS−/− may be due to the effect of deleting nNOS from other organs or a change in the control of neural innervation of the proximal tubule. In fact, it has been shown that the effects of NOS inhibition in proximal tubule transport are modulated by different degrees of neural activity (63, 108).

Cardiac function. In the heart, nNOS is found in the sarcoplasmic reticulum (109) and mitochondria of cardiac myocytes (42), cholinergic and nonadrenergic/noncholinergic nerve terminals, and in sympathetic nerve terminals, where it has been postulated to play a role in catecholamine release and reuptake (14, 15, 40). Although blood pressure is normal in nNOS−/−, studies have shown that this NOS isoform is important for maintenance of normal cardiac function. Basal heart rates have been found to be slightly increased in nNOS−/− (14, 40). It has also been reported that heart rate variability is decreased in nNOS−/−, suggesting that the increased heart rate may be due to reduced parasympathetic tone (40). In addition, atropine, a muscarinic antagonist, increased the heart rate in wild-type mice but had no effect in nNOS−/−, suggesting that muscarinic tone was already blunted (40). In agreement with nNOS controlling heart rate, Choate et al. (14) reported that vagal nerve stimulation caused a much slower decrease in nNOS−/− heart rate compared with wild-type mice, but the magnitude of the response was similar in both strains. The heart rate decrease caused by a muscarinic agonist was similar in both strains. These data suggest that vagal control of bradycardia is modulated by nNOS-derived NO via a presynaptic mechanism. The exact mechanism by which nNOS-derived NO modulates parasympathetic tone has not been fully studied to our knowledge.

The presence of nNOS in the sarcoplasmic reticulum of cardiac myocytes has led to the hypothesis that nNOS-derived NO modulates calcium fluxes and myocardial contractility (110). Data presented in two recent studies support this hypothesis. Ashley et al. (3) found that in isolated cardiac myocytes the percentage of cell shortening (an indicator of contractility) was enhanced in nNOS−/− myocytes during electrical stimulation. In addition, the contractile response to isoproterenol was enhanced in nNOS−/− myocytes. Similarly, Barouch et al. (4) found that in vivo basal cardiac contractility was enhanced in nNOS−/− as shown by increased dP/dt_{max}. However, when they studied the β-adrenergic response, they found decreased isoproterenol-stimulated contractility in nNOS−/− compared with wild-type mice. These authors also reported that isoproterenol-stimulated increases in intracellular calcium and contractility in isolated cardiac myocytes were almost completely abolished in nNOS−/−. These data suggest that nNOS-derived NO increases intracellular calcium and cardiac contractility, whereas eNOS-derived NO has the opposite effect. Interestingly, in double e-nNOS−/− mice basal cardiac contractility in vivo was even higher than in nNOS−/−, eNOS−/−, and wild-type hearts and isoproterenol-stimulated contractility was almost normal (4). Overall, the data suggest that under basal conditions both eNOS and nNOS decrease contractility; however, during β-adrenergic stimulation, eNOS decreases contractility while nNOS increases it (Fig. 3). As with other intracellular signaling molecules (e.g., cAMP), the effect of the second messenger is regulated by strictly controlling the site of production and the intracellular location of the target protein complexes (21, 39, 83). For NO signaling, these data suggest a new level of regulation that has not yet been studied.

iNOS−/−

Blood pressure. The gene locus coding for iNOS has been shown to cosegregate with blood pressure in the Dahl salt-sensitive rat (17). Whereas in most tissues iNOS is only induced by proinflammatory factors, it is expressed constitutively in the renal medulla (44). Despite the implication that iNOS may be involved in the development of salt-sensitive hypertension, there have been very few studies testing this hypothesis or questioning the role of iNOS in blood pressure regulation. Recently, Ihrig et al. (37) reported that basal systolic blood pressure was elevated by 10 mmHg in iNOS−/− at 3 mo of age, but was no different from wild-type mice at 9 or 12 mo. Feeding iNOS−/− a high-salt diet for 8 wk did not increase blood pressure further by 3 mo and had no effect at 9 to 12 mo. Interestingly, and similar to reports in eNOS−/− mice, iNOS−/− had higher plasma cholesterol levels than the wild type (19, 37). Ullrich et al. (97) also observed no differences in mean

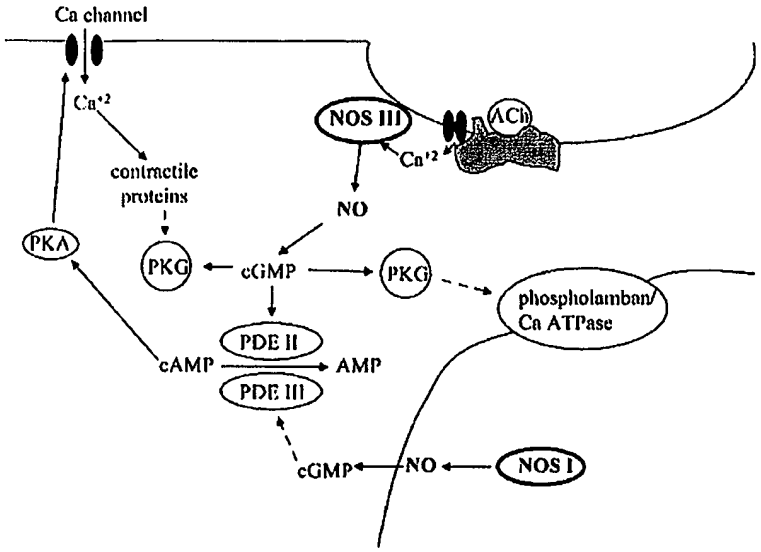

Fig. 3. Potential role of the different NOS isoforms in cardiac myocytes. PKG, protein kinase G; PDE II, cGMP-stimulated phosphodiesterase; PDE III, cGMP-inhibited phosphodiesterase; PKA, protein kinase A.

arterial pressure in 2- to 5-mo old iNOS−/−. We could find no other studies regarding blood pressure regulation in iNOS−/− under physiological conditions, nor of its relationship to age, salt sensitivity, and other vasoactive hormones. Thus more work in this area is needed.

Vascular function. iNOS expression has not been found in the vasculature under physiological conditions and thus is not likely to play a role in basal regulation of vascular tone. Only after its induction by inflammatory factors such as lipopolysaccharides, tumor necrosis factor, or interleukin-1 has iNOS-derived NO been shown to affect vascular tone (13, 88, 97). Given that iNOS induction appears to occur only under pathological conditions (i.e., septic shock) and there are few studies on its role in vascular tone regulation in iNOS−/− mice, it will not be discussed further here.

Renal function. In contrast to findings in other organs, iNOS is constitutively expressed in the renal medulla and proximal tubule (2, 44). However, the role of iNOS in the regulation of nephron transport has not been studied extensively. It was first reported that in proximal tubules stimulated with lipopolysaccharides, L-arginine decreased Na^+-K^+-ATPase activity, consistent with data showing that exogenous NO inhibits both apical Na^+ entry and basolateral Na^+ pump activity in this segment (26). In contrast to previous results, Wang (103) recently reported that in proximal tubules of iNOS−/− perfused in vivo, basal fluid and bicarbonate reabsorption were lower than in wild-type mice. They concluded that under basal conditions NO produced by iNOS in the proximal tubule stimulates solute and fluid reabsorption. There is currently no explanation for these disparate results, and more data are needed to resolve this issue.

Cardiac function. We know of no data regarding iNOS expression in mouse hearts under basal conditions. Ullrich et al. (97) reported that iNOS−/− mice have a heart rate similar to that of wild-type mice as well as normal blood pressure. It has been proposed that iNOS induction in the heart during chronic inflammation may lead to heart failure and other deleterious effects. However, it is still not defined whether iNOS induction is important to the development of heart failure or whether it is a consequence of the inflammatory response. Conflicting results have been obtained from experiments in which iNOS was overexpressed in cardiac myocytes. Heger et al. (29) found that iNOS overexpression caused a small decrease in heart rate and cardiac output but no other abnormalities in cardiac function, histology or anatomy. However, Mungrue et al. (58) found increased ventricular size, abnormal conduction, and increased mortality in these mice. Although the different results may be due to different levels of iNOS expression in these mice, the precise role of iNOS in the heart is still unclear.

CONCLUDING REMARKS

Genetic deletion of the various NOS isoforms has greatly aided our understanding of how NO and the three NOS isoforms regulate blood pressure and cardiovascular/renal function. However, many questions remain that cannot be answered with these models. Further clarification will require the development of inducible tissue-specific knockout of e, i, and nNOS. Additionally, the completion of the Human Genome Project has fundamentally changed the way we describe a gene and its function. It is now apparent that the concept of "one gene, one protein" was naive. Genetic deletion of a single gene may have physiologically significant effects in addition to, or more important than, those being studied. Thus the set of parameters we choose to study in knockout mice may not in fact reflect the most important physiological function of the absent gene.

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